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Research Article

Morphology and phylogeny of *Cytospora* (Cytosporaceae, Diaporthales) species associated with plant cankers in Tibet, China

Jiangrong Li[1,2], Jieting Li[1,2], Ning Jiang[3]

1 *Key Laboratory of Forest Ecology in Tibet Plateau, Ministry of Education,Institute of Tibet Plateau Ecology, Tibet Agricultual & Animal Husbandry University, Nyingchi, Tibet 860000, China*
2 *National Key Station of Field Scientific Observation & Experiment, Nyingchi, Tibet 860000, China*
3 *Key Laboratory of Biodiversity Conservation of National Forestry and Grassland Administration, Ecology and Nature Conservation Institute, Chinese Academy of Forestry, Beijing 100091, China*

Corresponding author: Ning Jiang (n.jiang@caf.ac.cn)

Abstract

During our biodiversity investigations in Tibet, China, typical Cytospora canker symptoms were observed on branches of hosts *Myricaria paniculate*, *Prunus cerasifera* and *Sibiraea angustata*. Samples were studied, based on morphological features coupled with multigene phylogenetic analyses of ITS, *act*, *rpb2*, *tef1* and *tub2* sequence data, which revealed two new species (*Cytospora myricicola* **sp. nov.** and *C. sibiraeicola* **sp. nov.**) and a known species (*C. populina*). In addition, *Cytospora populina* is newly discovered on the host *Prunus cerasifera* and in Tibet.

Key words: Ascomycota, molecular phylogeny, novel taxa, Sordariomycetes, taxonomy

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Introduction

The genus *Cytospora* (Cytosporaceae, Diaporthales, Sordariomycetes, Ascomycota) was proposed by Ehrenberg (1818) and *C. chrysosperma* was selected as the lectotype later (Donk 1964). *Cytospora* has priority over *Leucocytospora*, *Leucostoma*, *Valsa*, *Valsella* and *Valseutypella* based on the dual-nomenclature criterion (Adams et al. 2005; Rossman et al. 2015). Members of *Cytospora* are characterised by the single or labyrinthine, loculate stromata, filamentous conidiophores or asci and allantoid hyaline conidia or ascospores (Spielman 1985; Adams et al. 2005; Norphanphoun et al. 2017, 2018; Fan et al. 2020; Shang et al. 2020). Species identification in *Cytospora* was previously largely based on the host affiliation and morphological descriptions; however, molecular phylogeny combined with morphology and host affiliation have became the main approaches recently (Fan et al. 2020; Shang et al. 2020; Zhu et al. 2020). Currently, over 690 species epithets of *Cytospora* have been listed in Index Fungorum (http://www.indexfungorum.org/; 2023). However, most of these were regarded as synonyms and most descriptions were unable to identify them accurately (Adams et al. 2005; Fan et al. 2020; Pan et al. 2020).

Cytospora is distributed worldwide and often known to be associated with plant diseases (Monkai et al. 2021; Pan et al. 2021; Lin et al. 2022; Travadon et al. 2022).

For example, *C. chrysosperma* is the main canker disease pathogen of polar and willow trees in China (Fan et al. 2014; Wang et al. 2015; Lin et al. 2023); *C. kuanchengensis* and additional five species are associated with Chinese chestnut cankers (Jiang et al. 2020); Fifteen *Cytospora* species were identified from destructive canker and dieback pathogens of woody hosts in the USA (Lawrence et al. 2018).

The Tibet Tibetan Autonomous Region is located on the Qinghai-Tibet Plateau, which is known as the third pole of the earth. During our biodiversity investigations in Tibet, typical fruiting bodies of *Cytospora* were discovered from *Myricaria paniculate* (Tamaricaceae), *Prunus cerasifera* (Rosaceae) and *Sibiraea angustata* (Rosaceae). The aim of the present study was to identify *Cytospora* species from the three hosts based on morphological features and molecular phylogeny of combined sequence data.

Materials and methods

Sample collection, morphology and isolation

Our biodiversity investigations were conducted in Lhasa and Shigatse cities in Tibet Tibetan Autonomous Region, China during 2022 and 2023. Diseased branches of *Myricaria paniculate*, *Prunus cerasifera* and *Sibiraea angustata* were observed and collected, packed in paper bags and returned to the laboratory for morphological study and fungal isolation.

Observation and description of *Cytospora* species was based on fruiting bodies naturally formed on the host barks. Ascostromata and conidiomata from tree barks were sectioned by hand using a double-edged blade and structures were observed under a dissecting microscope. At least 10 conidiomata/ascostromata, 10 asci and 50 conidia/ascospores were measured to calculate the mean size and standard deviation. Measurements are reported as maximum and minimum in parentheses and the range representing the mean plus and minus the standard deviation of the number of measurements is given in parentheses. Microscopy photographs were captured with a Nikon Eclipse 80i compound microscope, equipped with a Nikon digital sight DS-Ri2 high definition colour camera, using differential interference contrast illumination.

Isolates of *Cytospora* were obtained by removing the spore masses from the fruiting bodies on to clean PDA plates and incubating at 25 °C until spores germinated. Single germinated spores were then transferred to the new PDA plates and incubated at 25 °C in the dark. The cultures were deposited in the China Forestry Culture Collection Center (CFCC, http://cfcc.caf.ac.cn/) and the specimens in the Herbarium of the Chinese Academy of Forestry (CAF, http://museum.caf.ac.cn/).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh fungal mycelia following the method described by Doyle and Doyle (1990). Polymerase chain reactions (PCR) were conducted to amplify the internal transcribed spacer region rDNA (ITS), the partial actin (*act*) region, RNA polymerase II second largest subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*) and the partial beta-tubulin (*tub2*) gene using primers and conditions listed in Table 1. The PCR products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL

Table 1. Primers and PCR protocols.

Gene Regions	Primers	PCR conditions	References
ITS	ITS5/ITS4	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 48 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	White et al. (1990)
act	ACT512F/ACT783R	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 55 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	Carbone and Kohn (1999)
rpb2	fRPB2-5f/fRPB2-7cR	95 °C for 5 min, 35 cycles of 95 °C for 1 min, 55 °C, 1.25 min, and 72 °C for 2 min , 72 °C for 10 min	Liu et al. (1999)
tef1	983F/2218R	94 °C for 3 min, 35 cycles of 94 °C for 30 s, 54 °C for 50 s, and 72 °C for 2 min, 72 °C for 10 min	Rehner (2001)
tub2	Bt2a/Bt2b	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 54 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	Glass and Donaldson (1995)

DNA Analyser with a BigDye Terminator Kit v.3.1 (Invitrogen, Waltham, MA, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Sequence alignment and phylogenetic analyses

The obtained sequences of ITS, *act*, *rpb2*, *tef1* and *tub2* were assembled using SeqMan software version 7.1.0 (DNASTAR Inc., WI) and subjected to BLASTn search against the GenBank nucleotide database at National Center for Biotechnology Information (NCBI) to identify closely-related sequences. Sequences data of related taxa were obtained from previous publications (Fan et al. 2020; Lin et al. 2023) and downloaded from the GenBank database (Table 2). The sequences were aligned using MAFFT v.7 online web server (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2019) under default settings. The Maximum Likelihood (ML) phylogenic analysis was run in the CIPRES Science Gateway platform (Miller et al. 2010), using RAxMLHPC2 on the XSEDE (v. 8.2.10) tool under the GTR substitution model and 1000 non-parametric bootstrap replicates. Bayesian analysis was performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES Science Gateway with four simultaneous Markov Chain runs for 1,000.000 generations. The resulting trees were visualised in FigTree v. 1.4.0 (Rambaut 2012).

Results

Phylogenetic analyses

The combined ITS, *act*, *rpb2*, *tef1* and *tub2* dataset consisted of 199 strains, with *Diaporthe vaccinii* (CBS 160.32) as the outgroup taxon (Table 2). The final alignment comprised 3,166 characters (ITS: 567, *act*: 323, *rpb2*: 741, *tef1*: 727, *tub2*: 808), including gaps. The final ML optimisation likelihood value of the best RAxML tree was -60353.67 and the matrix had 2069 distinct alignment patterns, with 40.24% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0. 244507, C = 0. 288246, G = 0. 237262, T = 0. 229984; substitution rates AC = 1.372283, AG = 2.995828, AT = 1.353835, CG = 0.976452, CT = 5.021434, GT = 1.0; gamma distribution shape parameter α = 0.372885. The RAxML and Bayesian analyses yielded a similar tree topology. The topology of our phylogenetic tree is nearly identical to previous publications. Six isolates from the present study formed two new clades distinct from previously-known species named *Cytospora myricicola* sp. nov. and *C. sibiraeicola* sp. nov. and a known clade named *C. populina* (Fig. 1).

Table 2. GenBank accession numbers used in the phylogenetic analyses.

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora ailanthicola	CFCC 89970	*Ailanthus altissima*	China	MH933618	MH933526	MH933592	MH933494	MH933565
Cytospora albodisca	CFCC 53161	*Platycladus orientalis*	China	MW418406	MW422899	MW422909	MW422921	MW422933
Cytospora albodisca	CFCC 54373	*Platycladus orientalis*	China	MW418407	MW422900	MW422910	MW422922	MW422934
Cytospora alba	CFCC 55462^{T}	*Salix matsudana*	China	NR182387	OK303457	OK303516	OK303577	OK303644
Cytospora alba	CFCC 55463	*Salix matsudana*	China	MZ702596	OK303458	OK303517	OK303578	OK303645
Cytospora ampulliformis	MFLUCC 16-0583^{T}	*Sorbus intermedia*	Russia	KY417726	KY417692	KY417794	NA	NA
Cytospora ampulliformis	MFLUCC 16-0629	*Acer platanoides*	Russia	KY417727	KY417693	KY417795	NA	NA
Cytospora amygdali	CBS 144233^{T}	*Prunus dulcis*	USA	MG971853	MG972002	NA	MG971659	NA
Cytospora atrocirrhata	CFCC 89615	*Juglans regia*	China	KR045618	KF498673	KU710946	KP310858	KR045659
Cytospora atrocirrhata	CFCC 89616	*Juglans regia*	China	KR045619	KF498674	KU710947	KP310859	KR045660
Cytospora beilinensis	CFCC 50493^{T}	*Pinus armandii*	China	MH933619	MH933527	NA	MH933495	MH933561
Cytospora beilinensis	CFCC 50494	*Pinus armandii*	China	MH933620	MH933528	NA	MH933496	MH933562
Cytospora berberidis	CFCC 89927^{T}	*Berberis dasystachya*	China	KR045620	KU710990	KU710948	KU710913	KR045661
Cytospora berberidis	CFCC 89933	*Berberis dasystachya*	China	KR045621	KU710991	KU710949	KU710914	KR045662
Cytospora bungeana	CFCC 50495^{T}	*Pinus bungeana*	China	MH933621	MH933529	MH933593	MH933497	MH933563
Cytospora bungeana	CFCC 50496	*Pinus bungeana*	China	MH933622	MH933530	MH933594	MH933498	MH933564
Cytospora californica	CBS 144234^{T}	*Juglans regia*	USA	MG971935	MG972083	NA	MG971645	NA
Cytospora carbonacea	CFCC 89947	*Ulmus pumila*	China	KR045622	KP310842	KU710950	KP310855	KP310825
Cytospora carpobroti	CMW48981^{T}	*Carpobrotus edulis*	South Africa	MH382812	NA	NA	MH411212	MH411207
Cytospora celtidicola	CFCC 50497^{T}	*Celtis sinensis*	China	MH933623	MH933531	MH933595	MH933499	MH933566
Cytospora celtidicola	CFCC 50498	*Celtis sinensis*	Anhui, China	MH933624	MH933532	MH933596	MH933500	MH933567
Cytospora centrivillosa	MFLUCC 16-1206^{T}	*Sorbus domestica*	Italy	MF190122	NA	MF377600	NA	NA
Cytospora centrivillosa	MFLUCC 17-1660	*Sorbus domestica*	Italy	MF190123	NA	MF377601	NA	NA
Cytospora ceratosperma	CFCC 89624	*Juglans regia*	China	KR045645	NA	KU710976	KP310860	KR045686
Cytospora ceratosperma	CFCC 89625	*Juglans regia*	China	KR045646	NA	KU710977	KP310861	KR045687
Cytospora ceratospermopsis	CFCC 89626^{T}	*Juglans regia*	China	KR045647	KU711011	KU710978	KU710934	KR045688
Cytospora ceratospermopsis	CFCC 89627	*Juglans regia*	China	KR045648	KU711012	KU710979	KU710935	KR045689
Cytospora chrysosperma	CFCC 89629	*Salix psammophila*	China	KF765673	NA	KF765705	NA	NA
Cytospora chrysosperma	CFCC 89981	*Populus alba*	China	MH933625	MH933533	MH933597	MH933501	MH933568
Cytospora chrysosperma	CFCC 89982	*Ulmus pumila*	China	KP281261	KP310835	NA	KP310848	KP310818
Cytospora cinnamomea	CFCC 53178^{T}	*Prunus armeniaca*	China	MK673054	MK673024	NA	NA	MK672970
Cytospora coryli	CFCC 53162^{T}	*Corylus mandshurica*	China	MN854450	NA	MN850751	MN850758	MN861120
Cytospora corylina	CFCC 54684^{T}	*Corylus heterophylla*	China	MW839861	MW815937	MW815951	MW815886	MW883969
Cytospora corylina	CFCC 54685	*Corylus heterophylla*	China	MW839862	MW815938	MW815952	MW815887	MW883970
Cytospora cotini	MFLUCC 14-1050^{T}	*Cotinus coggygria*	Russia	KX430142	NA	KX430144	NA	NA
Cytospora cotoneastricola	CF 20197027	*Cotoneaster* sp.	China	MK673072	MK673042	MK673012	MK672958	MK672988
Cytospora cotoneastricola	CF 20197028	*Cotoneaster* sp.	China	MK673073	MK673043	MK673013	MK672959	MK672989
Cytospora curvata	MFLUCC 15-0865^{T}	*Salix alba*	Russia	KY417728	KY417694	NA	NA	NA
Cytospora curvispora	CFCC 54000^{T}	*Corylus heterophylla*	China	MW839851	MW815931	MW815945	MW815880	MW883963
Cytospora curvispora	CFCC 54001	*Corylus heterophylla*	China	MW839853	MW815932	MW815946	MW815881	MW883964
Cytospora davidiana	CXY 1350^{T}	*Populus davidiana*	China	KM034870	NA	NA	NA	NA
Cytospora diopuiensis	CFCC 55479	*Euonymus japonicus*	China	OQ344753	OQ410625	OQ398735	OQ398762	OQ398791

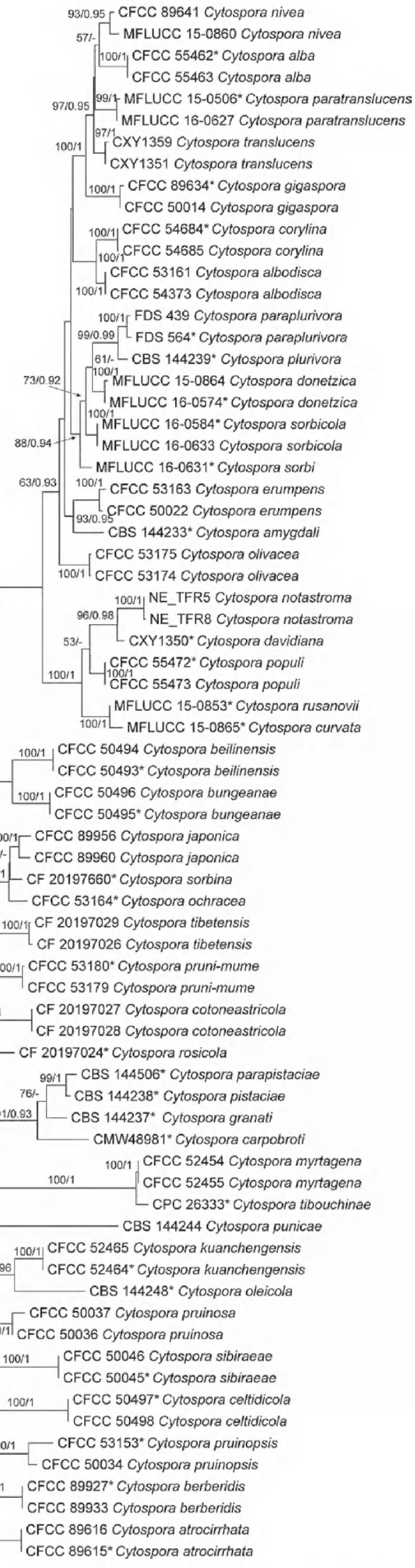
Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora diopuiensis	CFCC 55527	*Euonymus japonicus*	China	OQ344754	OQ410626	OQ398736	OQ398763	OQ398792
Cytospora discotoma	CFCC 53137^{T}	*Platycladus orientalis*	China	MW418404	MW422897	MW422907	MW422919	MW422931
Cytospora discotoma	CFCC 54368	*Platycladus orientalis*	China	MW418405	MW422898	MW422908	MW422920	MW422932
Cytospora donetzica	MFLUCC 15-0864	*Crataegus monogyna*	Russia	KY417729	KY417695	KY417797	NA	NA
Cytospora donetzica	MFLUCC 16-0574^{T}	*Crataegus monogyna*	Russia	KY417731	KY417697	KY417799	NA	NA
Cytospora donglingensis	CFCC 53159^{T}	*Platycladus orientalis*	China	MW418412	MW422903	MW422915	MW422927	MW422939
Cytospora donglingensis	CFCC 53160	*Platycladus orientalis*	China	MW418414	MW422905	MW422917	MW422929	MW422941
Cytospora elaeagni	CFCC 89632	*Elaeagnus angustifolia*	China	KR045626	KU710995	KU710955	KU710918	KR045667
Cytospora elaeagni	CFCC 89633	*Elaeagnus angustifolia*	China	KF765677	KU710996	KU710956	KU710919	KR045668
Cytospora elaeagnicola	CFCC 52882^{T}	*Elaeagnus angustifolia*	China	MK732341	MK732344	MK732347	NA	NA
Cytospora elaeagnicola	CFCC 52883	*Elaeagnus angustifolia*	China	MK732342	MK732345	MK732348	NA	NA
Cytospora erumpens	CFCC 50022	*Prunus padus*	China	MH933627	MH933534	NA	MH933502	MH933569
Cytospora erumpens	CFCC 53163	*Prunus padus*	China	MK673059	MK673029	MK673000	MK672948	MK672975
Cytospora eucalypti	CBS 144241	*Eucalyptus globulus*	USA	MG971907	MG972056	NA	MG971617	NA
Cytospora euonymicola	CFCC 50499^{T}	*Euonymus kiautschovicus*	China	MH933628	MH933535	MH933598	MH933503	MH933570
Cytospora euonymicola	CFCC 50500	*Euonymus kiautschovicus*	China	MH933629	MH933536	MH933599	MH933504	MH933571
Cytospora euonymina	CFCC 89993^{T}	*Euonymus kiautschovicus*	China	MH933630	MH933537	MH933600	MH933505	MH933590
Cytospora euonymina	CFCC 89999	*Euonymus kiautschovicus*	China	MH933631	MH933538	MH933601	MH933506	MH933591
Cytospora fraxinigena	MFLU 17-0880^{T}	*Fraxinus ornus*	Italy	NR154859	NA	NA	NA	NA
Cytospora fugax	CXY 1371	*Populus simonii*	China	KM034852	NA	NA	NA	KM034891
Cytospora fugax	CXY 1381	*Populus ussuriensis*	China	KM034853	NA	NA	NA	KM034890
Cytospora fusispora	NFCCI 4372	NA	India	MN227694	NA	NA	NA	NA
Cytospora galegicola	MFLUCC 18-1199^{T}	*Galega officinalis*	Italy	MK912128	MN685810	MN685820	NA	NA
Cytospora gigalocus	CFCC 89620^{T}	*Juglans regia*	China	KR045628	KU710997	KU710957	KU710920	KR045669
Cytospora gigalocus	CFCC 89621	*Juglans regia*	China	KR045629	KU710998	KU710958	KU710921	KR045670
Cytospora gigaspora	CFCC 50014	*Juniperus procumbens*	China	KR045630	KU710999	KU710959	KU710922	KR045671
Cytospora gigaspora	CFCC 89634^{T}	*Salix psammophila*	China	KF765671	KU711000	KU710960	KU710923	KR045672
Cytospora globosa	MFLU 16-2054^{T}	*Abies alba*	Italy	MT177935	NA	MT432212	MT454016	NA
Cytospora granati	CBS 144237^{T}	*Punica granatum*	USA	MG971799	MG971949	NA	MG971514	NA
Cytospora haidianensis	CFCC 54056	*Euonymus alatus*	China	MT360041	MT363978	MT363987	MT363997	MT364007
Cytospora haidianensis	CFCC 54057^{T}	*Euonymus alatus*	China	MT360042	MT363979	MT363988	MT363998	MT364008
Cytospora hippophaës	CFCC 89639	*Hippophaë rhamnoides*	China	KR045632	KU711001	KU710961	KU710924	KR045673
Cytospora hippophaës	CFCC 89640	*Hippophaë rhamnoides*	China	KF765682	KF765730	KU710962	KP310865	KR045674
Cytospora japonica	CFCC 89956	*Prunus cerasifera*	China	KR045624	KU710993	KU710953	KU710916	KR045665
Cytospora japonica	CFCC 89960	*Prunus cerasifera*	China	KR045625	KU710994	KU710954	KU710917	KR045666
Cytospora joaquinensis	CBS 144235	*Populus deltoides*	USA	MG971895	MG972044	NA	MG971605	NA
Cytospora junipericola	MFLU 17-0882^{T}	*Juniperus communis*	Italy	MF190125	NA	NA	MF377580	NA
Cytospora juniperina	CFCC 50501^{T}	*Juniperus przewalskii*	China	MH933632	MH933539	MH933602	MH933507	NA

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora juniperina	CFCC 50502	*Juniperus przewalskii*	China	MH933633	MH933540	MH933603	MH933508	MH933572
Cytospora kantschavelii	CXY 1383	*Populus maximowiczii*	China	KM034867	NA	NA	NA	NA
Cytospora kuanchengensis	CFCC 52464^{T}	*Castanea mollissima*	China	MK432616	MK442940	MK578076	NA	NA
Cytospora kuanchengensis	CFCC 52465	*Castanea mollissima*	China	MK432617	MK442941	MK578077	NA	NA
Cytospora leucosperma	CFCC 89622	*Pyrus bretschneideri*	China	KR045616	KU710988	KU710944	KU710911	KR045657
Cytospora leucosperma	CFCC 89894	*Pyrus bretschneideri*	China	KR045617	KU710989	KU710945	KU710912	KR045658
Cytospora longispora	CBS 144236^{T}	*Prunus domestica*	USA	MG971905	MG972054	NA	MG971615	NA
Cytospora longistiolata	MFLUCC 16-0628	*Salix* × *fragilis*	Russia	KY417734	KY417700	KY417802	NA	NA
Cytospora lumnitzericola	MFLUCC 17-0508^{T}	*Lumnitzera racernosa*	Tailand	MG975778	MH253457	MH253461	NA	NA
Cytospora mali	CFCC 50028	*Malus pumila*	China	MH933641	MH933548	MH933606	MH933513	MH933577
Cytospora mali	CFCC 50029	*Malus pumila*	China	MH933642	MH933549	MH933607	MH933514	MH933578
Cytospora mali-spectabilis	CFCC 53181^{T}	*Malus spectabilis*	China	MK673066	MK673036	MK673006	MK672953	MK672982
Cytospora melnikii	CFCC 89984	*Rhus typhina*	China	MH933644	MH933551	MH933609	MH933515	MH933580
Cytospora myricicola	**CFCC 59323^{T}**	***Myricaria paniculate***	**China**	**OR769868**	**OR767324**	**OR767338**	**OR767364**	**OR767351**
Cytospora myricicola	**CFCC 59324**	***Myricaria paniculate***	**China**	**OR769869**	**OR767325**	**OR767339**	**OR767365**	**OR767352**
Cytospora myricicola	**CFCC 59325**	***Myricaria paniculate***	**China**	**OR769870**	**OR767326**	**OR767340**	**OR767366**	**OR767353**
Cytospora myrtagena	CFCC 52454	*Castanea mollissima*	China	MK432614	MK442938	MK578074	NA	NA
Cytospora myrtagena	CFCC 52455	*Castanea mollissima*	China	MK432615	MK442939	MK578075	NA	NA
Cytospora nivea	MFLUCC 15-0860	*Salix acutifolia*	Russia	KY417737	KY417703	KY417805	NA	NA
Cytospora nivea	CFCC 89641	*Elaeagnus angustifolia*	China	KF765683	KU711006	KU710967	KU710929	KR045679
Cytospora notastroma	NE_TFR5	*Populus tremuloides*	USA	JX438632	NA	NA	JX438543	NA
Cytospora notastroma	NE_TFR8	*Populus tremuloides*	USA	JX438633	NA	NA	JX438542	NA
Cytospora ochracea	CFCC 53164^{T}	*Cotoneaster* sp.	China	MK673060	MK673030	MK673001	MK672949	MK672976
Cytospora oleicola	CBS 144248^{T}	*Olea europaea*	USA	MG971944	MG972098	NA	MG971660	NA
Cytospora olivacea	CFCC 53174	*Prunus cerasifera*	China	MK673058	MK673028	MK672999	NA	MK672974
Cytospora olivacea	CFCC 53175	*Prunus dulcis*	China	MK673062	MK673032	MK673003	NA	MK672978
Cytospora palm	CXY 1276	*Cotinus coggygria*	China	JN402990	NA	NA	KJ781296	NA
Cytospora palm	CXY 1280^{T}	*Cotinus coggygria*	China	JN411939	NA	NA	KJ781297	NA
Cytospora paracinnamomea	CFCC 55453^{T}	*Salix matsudana*	China	MZ702594	OK303456	OK303515	OK303576	OK303643
Cytospora paracinnamomea	CFCC 55455^{T}	*Salix matsudana*	China	MZ702598	OK303460	OK303519	OK303580	OK303647
Cytospora parakantschavelii	MFLUCC 15-0857^{T}	*Populus* × *sibirica*	Russia	KY417738	KY417704	KY417806	NA	NA
Cytospora parapistaciae	CBS 144506^{T}	*Pistacia vera*	USA	MG971804	MG971954	NA	MG971519	NA
Cytospora paraplurivora	FDS-439	*Prunus armeniaca*	Canada	OL640182	OL631586	NA	OL631589	NA
Cytospora paraplurivora	FDS-564^{T}	*Prunus persica* var. *nucipersica*	Canada	OL640183	OL631587	NA	OL631590	NA
Cytospora parasitica	CFCC 53173	*Berberis* sp.	China	MK673070	MK673040	MK673010	MK672957	MK672986
Cytospora paratranslucens	MFLUCC 15-0506^{T}	*Populus alba* var. *bolleana*	Russia	KY417741	KY417707	KY417809	NA	NA
Cytospora paratranslucens	MFLUCC 16-0627	*Populus alba*	Russia	KY417742	KY417708	KY417810	NA	NA
Cytospora phialidica	MFLUCC 17-2498	*Alnus glutinosa*	Italy	MT177932	NA	MT432209	MT454014	NA
Cytospora piceae	CFCC 52841^{T}	*Picea crassifolia*	China	MH820398	MH820406	MH820395	MH820402	MH820387
Cytospora piceae	CFCC 52842	*Picea crassifolia*	China	MH820399	MH820407	MH820396	MH820403	MH820388
Cytospora pingbianensis	MFLUCC 18-1204^{T}	Undefined wood	China	MK912135	MN685817	MN685826	NA	NA
Cytospora pistaciae	CBS 144238^{T}	*Pistacia vera*	USA	MG971802	MG971952	NA	MG971517	NA

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora platycladi	CFCC 50504^{T}	*Platycladus orientalis*	China	MH933645	MH933552	MH933610	MH933516	MH933581
Cytospora platycladi	CFCC 50505	*Platycladus orientalis*	China	MH933646	MH933553	MH933611	MH933517	MH933582
Cytospora platycladicola	CFCC 50038^{T}	*Platycladus orientalis*	China	KT222840	MH933555	MH933613	MH933519	MH933584
Cytospora platycladicola	CFCC 50039	*Platycladus orientalis*	China	KR045642	KU711008	KU710973	KU710931	KR045683
Cytospora plurivora	CBS 144239^{T}	*Olea europaea*	USA	MG971861	MG972010	NA	MG971572	NA
Cytospora populi	CFCC 55472^{T}	*Populus* sp.	China	MZ702609	OK303471	OK303530	OK303591	OK303658
Cytospora populi	CFCC 55473	*Populus* sp.	China	MZ702610	OK303472	OK303531	OK303592	OK303659
Cytospora populicola	CBS 144240	*Populus deltoides*	USA	MG971891	MG972040	NA	MG971601	NA
Cytospora populina	CFCC 89644^{T}	*Salix psammophila*	China	KF765686	KU711007	KU710969	KU710930	KR045681
Cytospora populina	**CFCC 58856**	***Prunus cerasifera***	**China**	**OR769873**	**OR767329**	**OR767343**	**OR767369**	**NA**
Cytospora populinopsis	CFCC 50032^{T}	*Sorbus aucuparia*	China	MH933648	MH933556	MH933614	MH933520	MH933585
Cytospora populinopsis	CFCC 50033	*Sorbus aucuparia*	China	MH933649	MH933557	MH933615	MH933521	MH933586
Cytospora predappioensis	MFLUCC 17-2458^{T}	*Platanus hybrida*	Italy	MG873484	NA	NA	NA	NA
Cytospora predappioensis	MFLU 17-0327	*Platanus hybrida*	Italy	MH253451	MH253449	MH253450	NA	NA
Cytospora prunicola	MFLU 17-0995^{T}	*Prunus* sp.	Italy	MG742350	MG742353	MG742352	NA	NA
Cytospora pruni-mume	CFCC 53179	*Prunus armeniaca*	China	MK673057	MK673027	NA	MK672947	MK672973
Cytospora pruni-mume	CFCC 53180^{T}	*Prunus mume*	China	MK673067	MK673037	MK673007	MK672954	MK672983
Cytospora pruinopsis	CFCC 50034^{T}	*Ulmus pumila*	China	KP281259	KP310836	KU710970	KP310849	KP310819
Cytospora pruinopsis	CFCC 53153	*Ulmus pumila*	China	MN854451	MN850763	MN850752	MN850759	MN861121
Cytospora pruinosa	CFCC 50036	*Syringa oblata*	China	KP310800	KP310832	NA	KP310845	KP310815
Cytospora pruinosa	CFCC 50037	*Syringa oblata*	China	MH933650	MH933558	NA	MH933522	MH933589
Cytospora pubescentis	MFLUCC 18-1201^{T}	*Quercus pubescens*	Italy	MK912130	MN685812	MN685821	NA	NA
Cytospora punicae	CBS 144244	*Punica granatum*	USA	MG971943	MG972091	NA	MG971654	NA
Cytospora quercicola	MFLU 17-0881	*Quercus* sp.	Italy	MF190128	NA	NA	NA	NA
Cytospora ribis	CFCC 50026	*Ulmus pumila*	China	KP281267	KP310843	KU710972	KP310856	KP310826
Cytospora ribis	CFCC 50027	*Ulmus pumila*	China	KP281268	KP310844	NA	KP310857	KP310827
Cytospora rosae	MFLU 17-0885	*Rosa canina*	Italy	MF190131	NA	NA	NA	NA
Cytospora rosicola	CF 20197024^{T}	*Rosa* sp.	China	MK673079	MK673049	MK673019	MK672965	MK672995
Cytospora rosigena	MFLUCC 18-0921^{T}	*Rosa* sp.	Russia	MN879872	NA	NA	NA	NA
Cytospora rostrata	CFCC 89909	*Salix cupularis*	China	KR045643	KU711009	KU710974	KU710932	KR045684
Cytospora rostrata	CFCC 89910	*Salix cupularis*	China	KR045644	KU711010	KU710975	KU710933	NA
Cytospora rusanovii	MFLUCC 15-0853	*Populus* × *sibirica*	Russia	KY417743	KY417709	KY417811	NA	NA
Cytospora rusanovii	MFLUCC 15-0854^{T}	*Salix babylonica*	Russia	KY417744	KY417710	KY417812	NA	NA
Cytospora salicacearum	MFLUCC 15-0509	*Salix alba*	Russia	KY417746	KY417712	KY417814	NA	NA
Cytospora salicacearum	MFLUCC 15-0861	*Salix* × *fragilis*	Russia	KY417745	KY417711	KY417813	NA	NA
Cytospora salicicola	MFLUCC 14-1052^{T}	*Salix alba*	Russia	KU982636	KU982637	NA	NA	NA
Cytospora salicicola	MFLUCC 15-0866	*Salix* sp.	Thailand	KY417749	KY417715	KY417817	NA	NA
Cytospora salicina	MFLUCC 15-0862	*Salix alba*	Russia	KY417750	KY417716	KY417818	NA	NA
Cytospora salicina	MFLUCC 16-0637	*Salix* × *fragilis*	Russia	KY417751	KY417717	KY417819	NA	NA
Cytospora schulzeri	CFCC 50042	*Malus pumila*	China	KR045650	KU711014	KU710981	KU710937	KR045691
Cytospora sibiraeae	CFCC 50045^{T}	*Sibiraea angustata*	China	KR045651	KU711015	KU710982	KU710938	KR045692
Cytospora sibiraeae	CFCC 50046	*Sibiraea angustata*	China	KR045652	KU711016	KU710983	KU710939	KR045693
Cytospora sibiraeicola	**CFCC 59100^{T}**	***Sibiraea angustata***	**China**	**OR769871**	**OR767327**	**OR767341**	**OR767367**	**OR767354**
Cytospora sibiraeicola	**CFCC 59101**	***Sibiraea angustata***	**China**	**OR769872**	**OR767328**	**OR767342**	**OR767368**	**OR767355**
Cytospora sophorae	CFCC 50047	*Styphnolobium japonicum*	China	KR045653	KU711017	KU710984	KU710940	KR045694

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora sophorae	CFCC 89598	*Styphnolobium japonicum*	China	KR045654	KU711018	KU710985	KU710941	KR045695
Cytospora sophoricola	CFCC 89596	*Styphnolobium japonicum*	China	KR045656	KU711020	KU710987	KU710943	KR045697
Cytospora sophoricola	CFCC 89595^{T}	*Styphnolobium japonicum*	China	KR045655	KU711019	KU710986	KU710942	KR045696
Cytospora sophoriopsis	CFCC 55469	*Salix matsudana*	China	MZ702583	OK303445	OK303504	OK303565	OK303632
Cytospora sophoriopsis	CFCC 89600	*Styphnolobium japonicum*	China	KR045623	KU710992	KU710951	KU710915	KP310817
Cytospora sorbi	MFLUCC 16-0631^{T}	*Sorbus aucuparia*	Russia	KY417752	KY417718	KY417820	NA	NA
Cytospora sorbicola	MFLUCC 16-0584^{T}	*Acer pseudoplatanus*	Russia	KY417755	KY417721	KY417823	NA	NA
Cytospora sorbicola	MFLUCC 16-0633	*Cotoneaster melanocarpus*	Russia	KY417758	KY417724	KY417826	NA	NA
Cytospora sorbina	CF 20197660^{T}	*Sorbus tianschanica*	China	MK673052	MK673022	NA	MK672943	MK672968
Cytospora spiraeae	CFCC 50049^{T}	*Spiraea salicifolia*	China	MG707859	MG708196	MG708199	NA	NA
Cytospora spiraeae	CFCC 50050	*Spiraea salicifolia*	China	MG707860	MG708197	MG708200	NA	NA
Cytospora spiraeicola	CFCC 53138^{T}	*Spiraea salicifolia*	China	MN854448	NA	MN850749	MN850756	MN861118
Cytospora spiraeicola	CFCC 53139	*Tilia nobilis*	China	MN854449	NA	MN850750	MN850757	MN861119
Cytospora tamaricicola	CFCC 50507	*Rosa multifolora*	China	MH933651	MH933559	MH933616	MH933525	MH933587
Cytospora tamaricicola	CFCC 50508^{T}	*Tamarix chinensis*	China	MH933652	MH933560	MH933617	MH933523	MH933588
Cytospora tanaitica	MFLUCC 14-1057^{T}	*Betula pubescens*	Russia	KT459411	KT459413	NA	NA	NA
Cytospora thailandica	MFLUCC 17-0262^{T}	*Xylocarpus moluccensis*	Thailand	MG975776	MH253459	MH253463	NA	NA
Cytospora thailandica	MFLUCC 17-0263^{T}	*Xylocarpus moluccensis*	Thailand	MG975777	MH253460	MH253464	NA	NA
Cytospora tibetensis	CF 20197026	*Cotoneaster* sp.	China	MK673076	MK673046	MK673016	MK672962	MK672992
Cytospora tibetensis	CF 20197029	*Cotoneaster* sp.	China	MK673077	MK673047	MK673017	MK672963	MK672993
Cytospora tibouchinae	CPC 26333^{T}	*Tibouchina semidecandra*	France	KX228284	NA	NA	NA	NA
Cytospora translucens	CXY 1351	*Populus davidiana*	China	KM034874	NA	NA	NA	KM034895
Cytospora translucens	CXY 1359	*Populus* × beijingensis	China	KM034871	NA	NA	NA	KM034894
Cytospora ulmi	MFLUCC 15-0863^{T}	*Ulmus minor*	Russia	KY417759	NA	NA	NA	NA
Cytospora verrucosa	CFCC 53157^{T}	*Platycladus orientalis*	China	MW418408	NA	MW422911	MW422923	MW422935
Cytospora verrucosa	CFCC 53158	*Platycladus orientalis*	China	MW418410	MW422901	MW422913	MW422925	MW422937
Cytospora vinacea	CBS 141585^{T}	*Vitis interspecific*	USA	KX256256	NA	NA	KX256277	KX256235
Cytospora viridistroma	CBS 202.36^{T}	*Cercis canadensis*	USA	MN172408	NA	NA	MN271853	NA
Cytospora viticola	Cyt2	*Vitis interspecific*	USA	KX256238	NA	NA	KX256259	KX256217
Cytospora viticola	CBS 141586^{T}	*Vitis vinifera*	USA	KX256239	NA	NA	KX256260	KX256218
Cytospora xinjiangensis	CFCC 53182	*Rosa* sp.	China	MK673064	MK673034	MK673004	MK672951	MK672980
Cytospora xinjiangensis	CFCC 53183^{T}	*Rosa* sp.	China	MK673065	MK673035	MK673005	MK672952	MK672981
Cytospora xinglongensis	CFCC 52458	*Castanea mollissima*	China	MK432622	MK442946	MK578082	NA	NA
Cytospora xinglongensis	CFCC 52459	*Castanea mollissima*	China	MK432623	MK442947	MK578083	NA	NA
Cytospora xylocarpi	MFLUCC 17-0251^{T}	*Xylocarpus granatum*	Thailand	MG975775	MH253458	MH253462	NA	NA
Cytospora zhaitangensis	CFCC 56227^{T}	*Euonymus japonicus*	China	OQ344750	OQ410623	OQ398733	OQ398760	OQ398789
Cytospora zhaitangensis	CFCC 57537	*Euonymus japonicus*	China	OQ344751	OQ410624	OQ398734	OQ398761	OQ398790
Diaporthe vaccinii	CBS 160.32	*Vaccinium macrocarpon*	USA	KC343228	JQ807297	NA	KC343954	KC344196

Ex-type strains are indicated with (^{T}) after the collection number; "NA" indicates unavailable sequences; sequences produced in the current study are in bold.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CFCC 55463 Cytospora alba
99/1 MFLUCC 15-0506* Cytospora paratranslucens
97/0.95 MFLUCC 16-0627 Cytospora paratranslucens
97/1 CXY1359 Cytospora translucens
100/1 CXY1351 Cytospora translucens
100/1 CFCC 89634* Cytospora gigaspora
CFCC 50014 Cytospora gigaspora
100/1 CFCC 54684* Cytospora corylina
100/1 CFCC 54685 Cytospora corylina
CFCC 53161 Cytospora albodisca
100/1 CFCC 54373 Cytospora albodisca
100/1 FDS 439 Cytospora paraplurivora
99/0.99 FDS 564* Cytospora paraplurivora
61/- CBS 144239* Cytospora plurivora
73/0.92 100/1 MFLUCC 15-0864 Cytospora donetzica
MFLUCC 16-0574* Cytospora donetzica
100/1 MFLUCC 16-0584* Cytospora sorbicola
88/0.94 MFLUCC 16-0633 Cytospora sorbicola
MFLUCC 16-0631* Cytospora sorbi
63/0.93 100/1 CFCC 53163 Cytospora erumpens
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93/0.95 CBS 144233* Cytospora amygdali
CFCC 53175 Cytospora olivacea
100/1 CFCC 53174 Cytospora olivacea
100/1 100/1 NE_TFR5 Cytospora notastroma
96/0.98 NE_TFR8 Cytospora notastroma
53/- CXY1350* Cytospora davidiana
CFCC 55472* Cytospora populi
57/- 100/1 100/1 CFCC 55473 Cytospora populi
MFLUCC 15-0853* Cytospora rusanovii
100/1 MFLUCC 15-0865* Cytospora curvata
100/1 CFCC 50494 Cytospora beilinensis
100/1 CFCC 50493* Cytospora beilinensis
100/1 CFCC 50496 Cytospora bungeanae
CFCC 50495* Cytospora bungeanae
100/1 CFCC 89956 Cytospora japonica
50/- CFCC 89960 Cytospora japonica
100/1 CF 20197660* Cytospora sorbina
56/- 75/0.96 CFCC 53164* Cytospora ochracea
100/1 CF 20197029 Cytospora tibetensis
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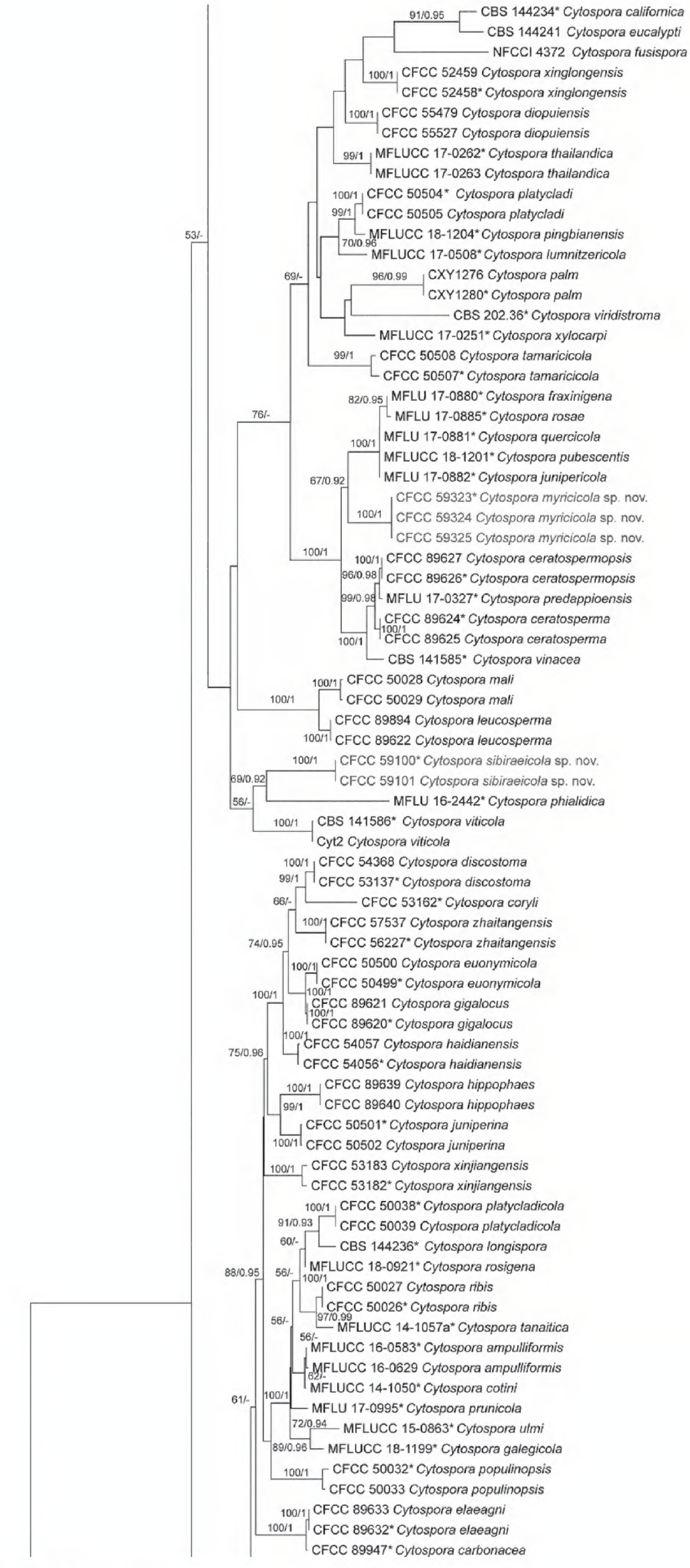
100/1 100/1 CFCC 53180* Cytospora pruni-mume
CFCC 53179 Cytospora pruni-mume
86/0.95 100/1 CF 20197027 Cytospora cotoneastricola
CF 20197028 Cytospora cotoneastricola
CF 20197024* Cytospora rosicola
99/1 CBS 144506* Cytospora parapistaciae
76/- CBS 144238* Cytospora pistaciae
91/0.93 CBS 144237* Cytospora granati
CMW48981* Cytospora carpobroti
93/0.95 100/1 CFCC 52454 Cytospora myrtagena
93/0.95 100/1 CFCC 52455 Cytospora myrtagena
CPC 26333* Cytospora tibouchinae
CBS 144244 Cytospora punicae
100/1 CFCC 52465 Cytospora kuanchengensis
70/0.96 CFCC 52464* Cytospora kuanchengensis
75/0.96 CBS 144248* Cytospora oleicola
CFCC 50037 Cytospora pruinosa
100/1 CFCC 50036 Cytospora pruinosa
100/1 CFCC 50046 Cytospora sibiraeae
CFCC 50045* Cytospora sibiraeae
86/0.98 100/1 CFCC 50497* Cytospora celtidicola
CFCC 50498 Cytospora celtidicola
100/1 CFCC 53153* Cytospora pruinopsis
CFCC 50034 Cytospora pruinopsis
100/1 CFCC 89927* Cytospora berberidis
CFCC 89933 Cytospora berberidis
100/1 CFCC 89616 Cytospora atrocirrhata
CFCC 89615* Cytospora atrocirrhata

Figure 1. Maximum Likelihood tree generated from combined ITS, *act*, *rpb2*, *tef1* and *tub2* sequence data. Bootstrap support values ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are demonstrated at the branches. Ex-type cultures are marked with (*).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NFCCI 4372 Cytospora fusispora
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CFCC 55479 Cytospora diopuiensis
CFCC 55527 Cytospora diopuiensis
MFLUCC 17-0262* Cytospora thailandica
MFLUCC 17-0263 Cytospora thailandica
CFCC 50504* Cytospora platycladi
CFCC 50505 Cytospora platycladi
MFLUCC 18-1204* Cytospora pingbianensis
MFLUCC 17-0508* Cytospora lumnitzericola
CXY1276 Cytospora palm
CXY1280* Cytospora palm
CBS 202.36* Cytospora viridistroma
MFLUCC 17-0251* Cytospora xylocarpi
CFCC 50508 Cytospora tamaricicola
CFCC 50507* Cytospora tamaricicola
MFLU 17-0880* Cytospora fraxinigena
MFLU 17-0885* Cytospora rosae
MFLU 17-0881* Cytospora quercicola
MFLUCC 18-1201* Cytospora pubescentis
MFLU 17-0882* Cytospora junipericola
CFCC 59323* Cytospora myricicola sp. nov.
CFCC 59324 Cytospora myricicola sp. nov.
CFCC 59325 Cytospora myricicola sp. nov.
CFCC 89627 Cytospora ceratospermopsis
CFCC 89626* Cytospora ceratospermopsis
MFLU 17-0327* Cytospora predappioensis
CFCC 89624* Cytospora ceratosperma
CFCC 89625 Cytospora ceratosperma
CBS 141585* Cytospora vinacea
CFCC 50028 Cytospora mali
CFCC 50029 Cytospora mali
CFCC 89894 Cytospora leucosperma
CFCC 89622 Cytospora leucosperma
CFCC 59100* Cytospora sibiraeicola sp. nov.
CFCC 59101 Cytospora sibiraeicola sp. nov.
MFLU 16-2442* Cytospora phialidica
CBS 141586* Cytospora viticola
Cyt2 Cytospora viticola
CFCC 54368 Cytospora discostoma
CFCC 53137* Cytospora discostoma
CFCC 53162* Cytospora coryli
CFCC 57537 Cytospora zhaitangensis
CFCC 56227* Cytospora zhaitangensis
CFCC 50500 Cytospora euonymicola
CFCC 50499* Cytospora euonymicola
CFCC 89621 Cytospora gigalocus
CFCC 89620* Cytospora gigalocus
CFCC 54057 Cytospora haidianensis
CFCC 54056* Cytospora haidianensis
CFCC 89639 Cytospora hippophaes
CFCC 89640 Cytospora hippophaes
CFCC 50501* Cytospora juniperina
CFCC 50502 Cytospora juniperina
CFCC 53183 Cytospora xinjiangensis
CFCC 53182* Cytospora xinjiangensis
CFCC 50038* Cytospora platycladicola
CFCC 50039 Cytospora platycladicola
CBS 144236* Cytospora longispora
MFLUCC 18-0921* Cytospora rosigena
CFCC 50027 Cytospora ribis
CFCC 50026* Cytospora ribis
MFLUCC 14-1057a* Cytospora tanaitica
MFLUCC 16-0583* Cytospora ampulliformis
MFLUCC 16-0629 Cytospora ampulliformis
MFLUCC 14-1050* Cytospora cotini
MFLU 17-0995* Cytospora prunicola
MFLUCC 15-0863* Cytospora ulmi
MFLUCC 18-1199* Cytospora galegicola
CFCC 50032* Cytospora populinopsis
CFCC 50033 Cytospora populinopsis
CFCC 89633 Cytospora elaeagni
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CFCC 89947* Cytospora carbonacea

Figure 1. Continued.

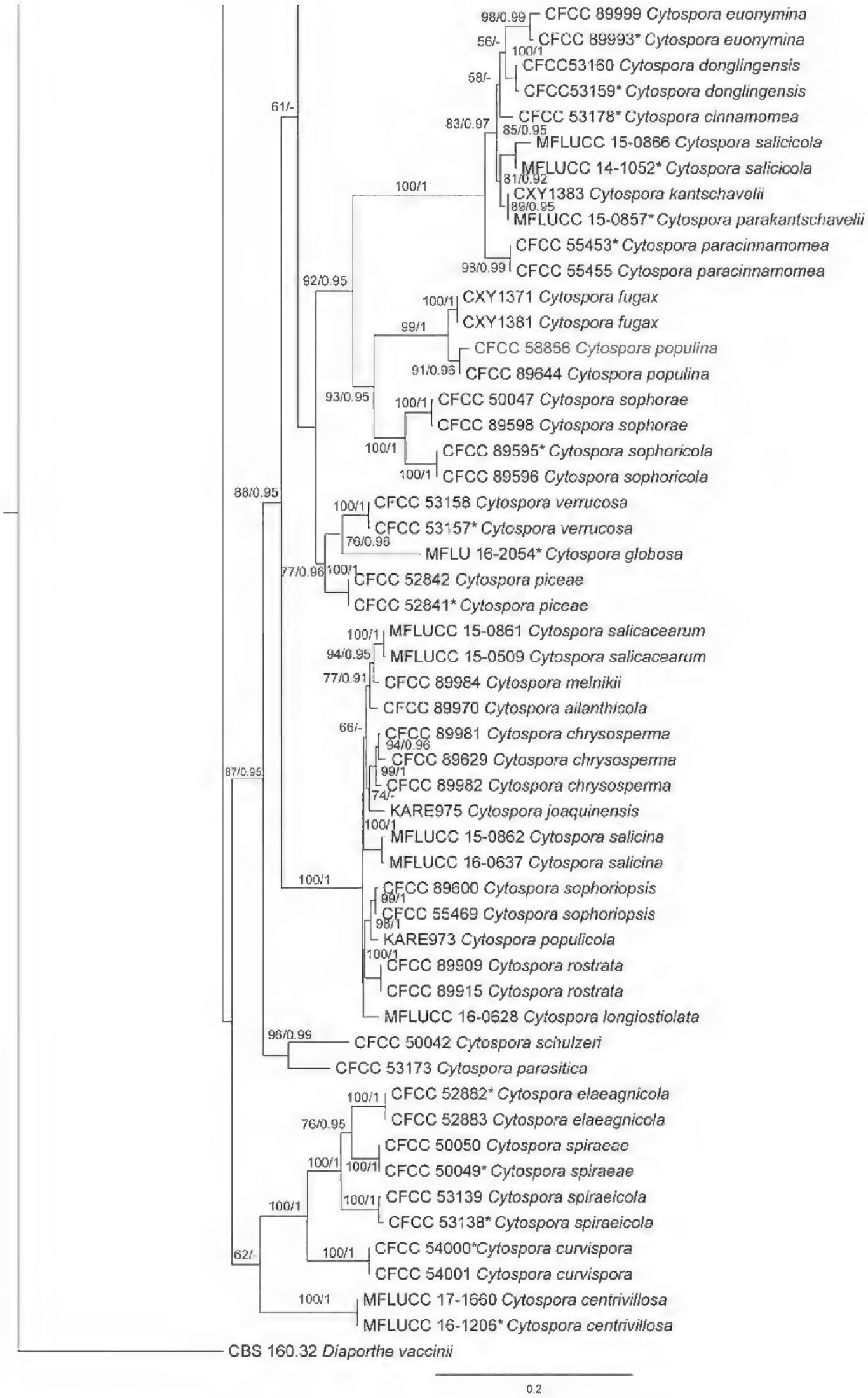

Figure 1. Continued.

Taxonomy

***Cytospora myricicola* Ning Jiang, sp. nov.**

MycoBank No: 850240

Fig. 2

Etymology. "*myrici*" refers to the host genus *Myricaria* and "*-cola*" means inhabiting.

Holotype. CAF800083.

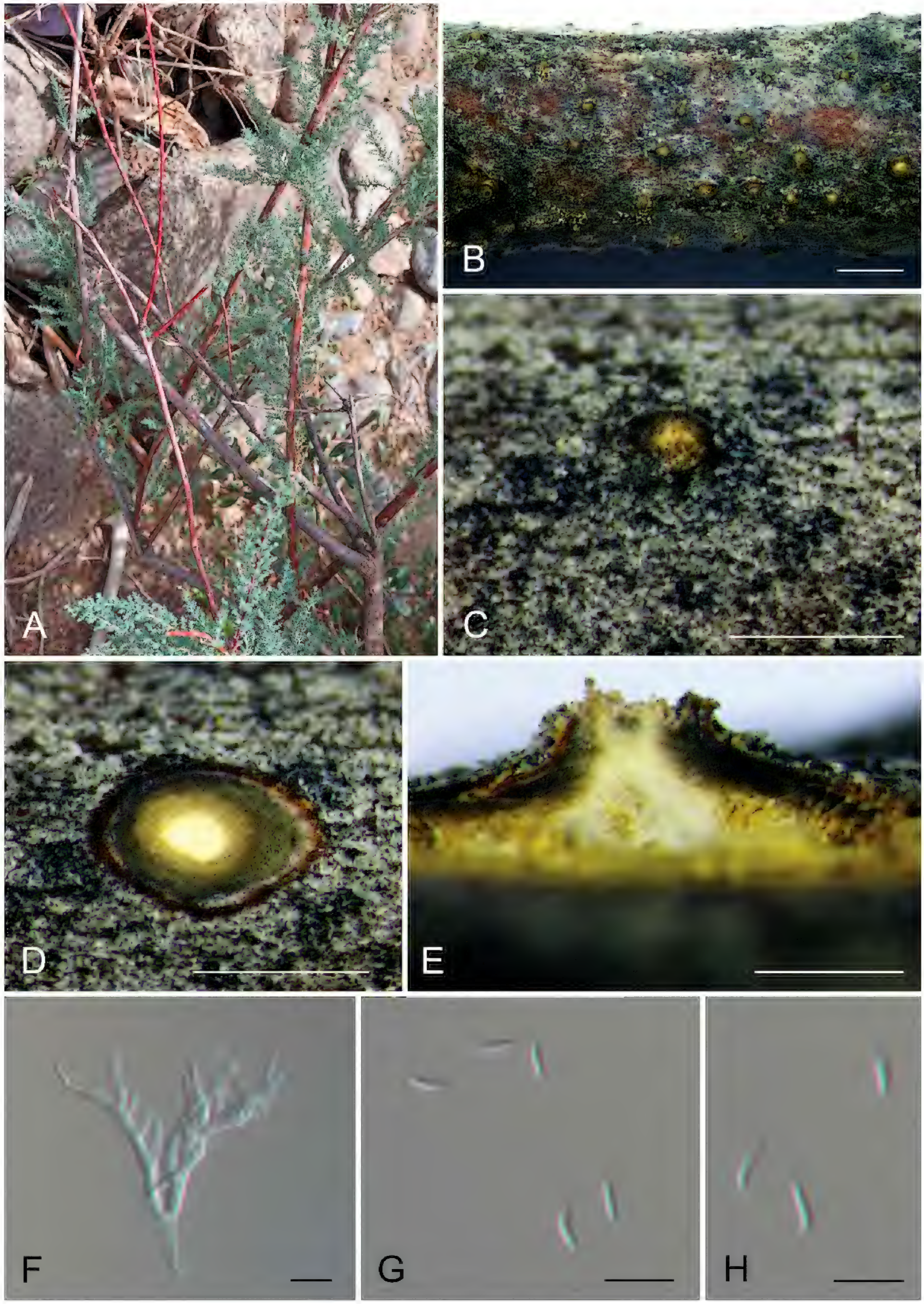

Figure 2. *Cytospora myricicola* (CAF800083, holotype) **A, B** canker disease symptom **C** conidioma **D** transverse section through a conidioma **E** longitudinal section through a conidioma **F** conidiophores and conidia **G, H** conidia. Scale bars: 2000 µm (**B**); 1000 µm (**C, D**); 500 µm (**E**); 10 µm (**F–H**).

Description. Associated with branch canker disease of *Myricaria paniculate*. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Pycnidial stromata ostiolated, semi-immersed in the host bark, scattered, discoid, with multiple locules. Conceptacle dark brown to black, circular surrounded stromata. Ectostromatic disc dark yellow, circular to ovoid, (250–)350–450(–550) µm diam., with one ostiole per disc. Ostioles dark, at the same level as the disc, (35–)55–85(–100) µm diam. Locule numerous, arranged circularly or elliptically with independent walls (245–)300–450(–550) µm diam. Peridium comprising a few layers of cells of textura angularis, with innermost layer brown, outer layer brown to dark brown. Conidiophores hyaline, branched, thin-walled, filamentous. Conidiogenous cells enteroblastic polyphialidic, 6.5–35.5 × 1.5–2.5 µm. Conidia hyaline, allantoid, smooth, aseptate, thin-walled, (4.4–)4.7–5.6(–5.8) × 1.4–1.7 µm ($\bar{x}$ = 5.2 × 1.6 µm).

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and entire edge, initially white, becoming dark and reaching 90 mm diameter after 10 days at 25 °C, sterile.

Materials examined. CHINA, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Zen Village, on cankered branches of *Myricaria paniculate*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (CAF800083, ***holotype***); ex-type culture CFCC 59323. China, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Renqinglin Village, on cankered branches of *Myricaria paniculate*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (XZ010B); cultures CFCC 59324 and CFCC 59325.

Notes. Phylogenetically, *Cytospora myricicola* is close to *C. fraxinigena*, *C. junipericola*, *C. pubescentis*, *C. quercicola* and *C. rosae* (Fig. 1). Of these six species, only *C. myricicola*, *C. pubescentis* and *C. rosae* have asexual morph descriptions; *C. myricicola* (4.7–5.6 × 1.4–1.7 µm) is different from *C. pubescentis* (5.8–7.5 × 1.3–1.6 µm) by shorter conidia and from *C. rosae* (3–5 × 0.5–1 µm) by larger conidia (Senanayake et al. 2017; Shang et al. 2020). In addition, *C. myricicola* can be distinguished from the other five species by host and distribution (*C. myricicola* from *Myricaria paniculate* in China vs. *C. fraxinigena* from *Fraxinus ornus* in Italy vs. *C. junipericola* from *Juniperus communis* in Italy vs. *C. pubescentis* from *Quercus pubescens* in Italy vs. *C. quercicola* from *Quercus* sp. in Italy vs. *C.* rosae from *Rosa canina* in Italy) (Senanayake et al. 2017; Shang et al. 2020).

***Cytospora populina* (Pers.) Rabenh., Deutschl. Krypt.-Fl. (Leipzig) 1: 148. 1844**
Fig. 3

Description. Associated with branch canker disease of *Prunus cerasifera*. ***Sexual morph*:** Stromata immersed in bark. Ascostromata, erumpent through the surface of bark, lenticular, extending to a large circular area, (750–)900–1200(–1350) µm diam. Disc grey to black, circular to ovoid, (85–)100–150(–195) µm in diameter. Ostioles numerous, dark brown to black, at the same level as the disc, (25–)31–46(–52) µm diam. Locules dark brown, arranged circularly, flask-shaped to spherical, (180–)195–285(–340) µm diam. Asci clavate to elongate obovoid, (45–)55.5–62.5(–67) × (6.5–)8–12(–16) µm, 4-spored. Ascospores

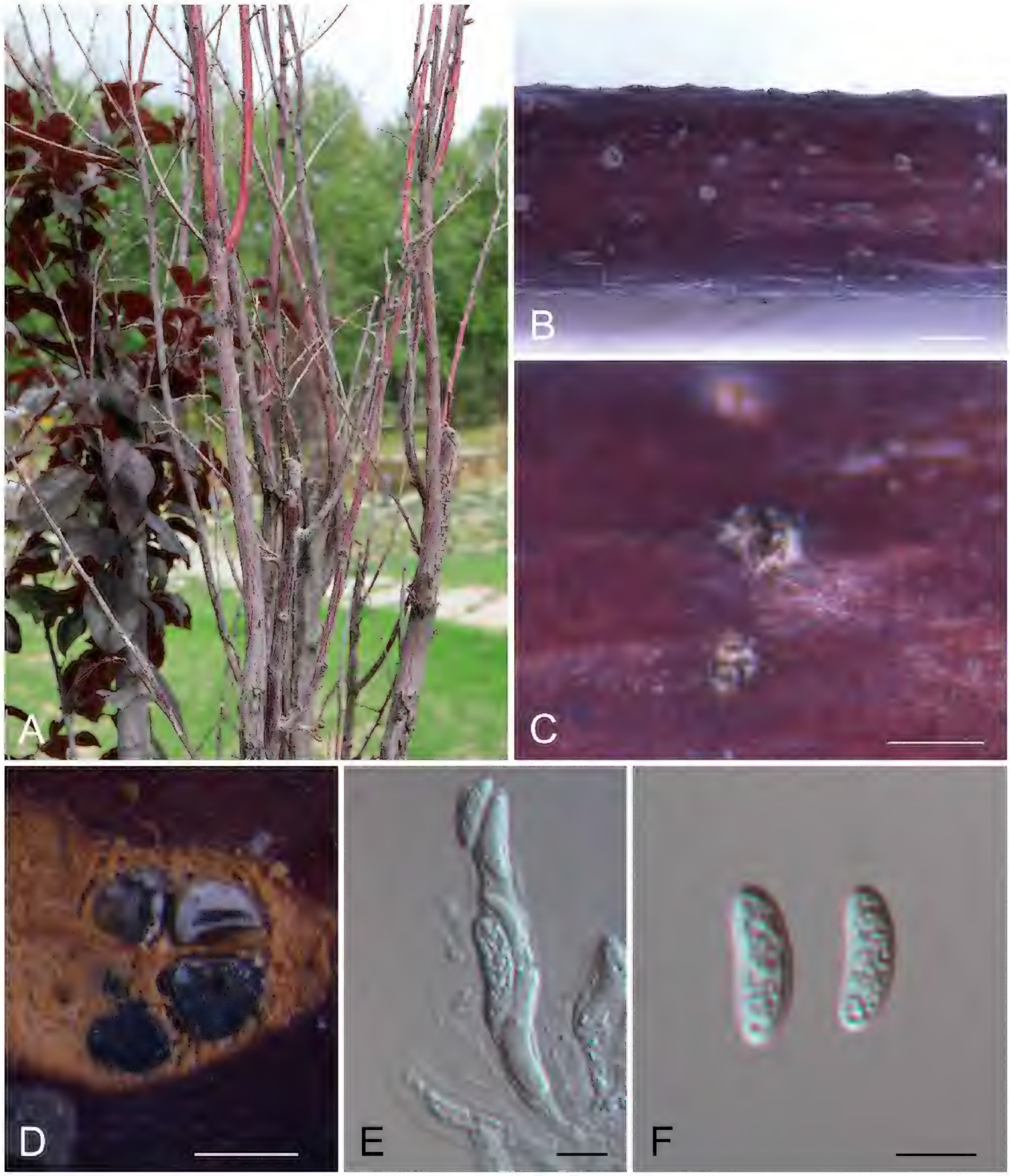

Figure 3. *Cytospora populina* (CAF800085) **A, B** canker disease symptom **C** ascostromata **D** transverse section through an ascostroma **E** asci and Ascospores **F** ascospores. Scale bars: 2000 µm (**B**); 500 µm (**C**); 200 µm (**D**); = 10 µm (**E–F**).

biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, (15–)18.5–23.5(–25.5) × (4–)4.5–5.5(–6.5) µm ($\bar{x}$ = 20.4 × 5.1 µm). ***Asexual morph*:** Undetermined.

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and entire edge, initially white, becoming luteous and reaching 80 mm diameter after 10 days at 25 °C, sterile.

Materials examined. CHINA, Tibet Tibetan Autonomous Region, Shigatse City, Sangzhuzi District, Gongjuelinka Park, on cankered branches of *Prunus cerasifera*, 2 August 2022, Jin Peng, Jiang Ning and Liu Min (XZ063); culture CFCC 58856.

Notes. *Cytospora populina* has been reported from *Populus canadensis* in Argentina, *Salix psammophila* in Shaanxi Province of China and *Acer pubescens Rubus* sp. in Uzbekistan (Farr 1973; Fan et al. 2015; Gafforov 2017). This fungus is distinguished from the other *Cytospora* species by its 4-ascospored asci and undiscovered asexual state (Fan et al. 2015). In the present study, we firstly found this fungus causing cankered branches of *Prunus cerasifera* in Tibet, China.

Cytospora sibiraeicola **Ning Jiang, sp. nov.**

MycoBank No: 850241

Fig. 4

Etymology. "*sibiraei*" refers to the host genus *Sibiraea* and "*-cola*" means inhabiting.

Holotype. CAF800084.

Description. Associated with branch canker disease of *Sibiraea angustata*. ***Sexual morph*****:** Undetermined. ***Asexual morph*****:** Pycnidial stromata ostiolated, immersed or semi-immersed in the host bark, scattered, discoid, with multiple locules. Conceptacle black, circular surrounded stromata. Ectostromatic disc black, circular to ovoid, (200–)300–450(–500) µm diam., with one ostiole per disc. Ostioles dark, at the same level as the disc, (30–)60–80(–95) µm diam. Locule numerous, arranged circularly or elliptically with independent walls, (200–)250–380(–500) µm diam. Peridium comprising few layers of cells of textura angularis, with innermost layer brown, outer layer brown to dark brown. Conidiophores hyaline, unbranched, thin-walled, filamentous. Conidiogenous cells enteroblastic polyphialidic, 12.5–32.5 × 2–3.5 µm. Conidia hyaline, allantoid, smooth, aseptate, thin-walled, (3.3–)3.4–4.3(–4.5) × 1.2–1.6 µm ($\bar{x}$ = 3.9 × 1.5 µm).

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and undulate margin, initially white, becoming olivaceous grey and reaching 90 mm diameter after 10 days at 25 °C, sterile.

Materials examined. CHINA, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Zen Village, on cankered branches of *Sibiraea angustata*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (CAF800083, holotype); ex-type cultures CFCC 59100 and CFCC 59101.

Notes. *Cytospora sibiraeicola* is phylogenetically close to *C. phialidica* and *C. viticola* (Fig. 1). Morphologically, *C. sibiraeicola* (3.4–4.3 × 1.2–1.6 µm) and *C. phialidica* (3.5–5 × 1–2 µm) have much shorter conidia than *C. viticola* (5.2–7 × 0.9–1.6 µm) (Lawrence et al. 2017; Li et al. 2020). In addition, these three species can be distinguished by the host and distribution (*C. sibiraeicola* from *Sibiraea angustata* in China vs. *C. phialidica* from *Alnus glutinosa* in Italy vs. *C. viticola* from *Vitis vinifera* in the USA) (Lawrence et al. 2017; Li et al. 2020).

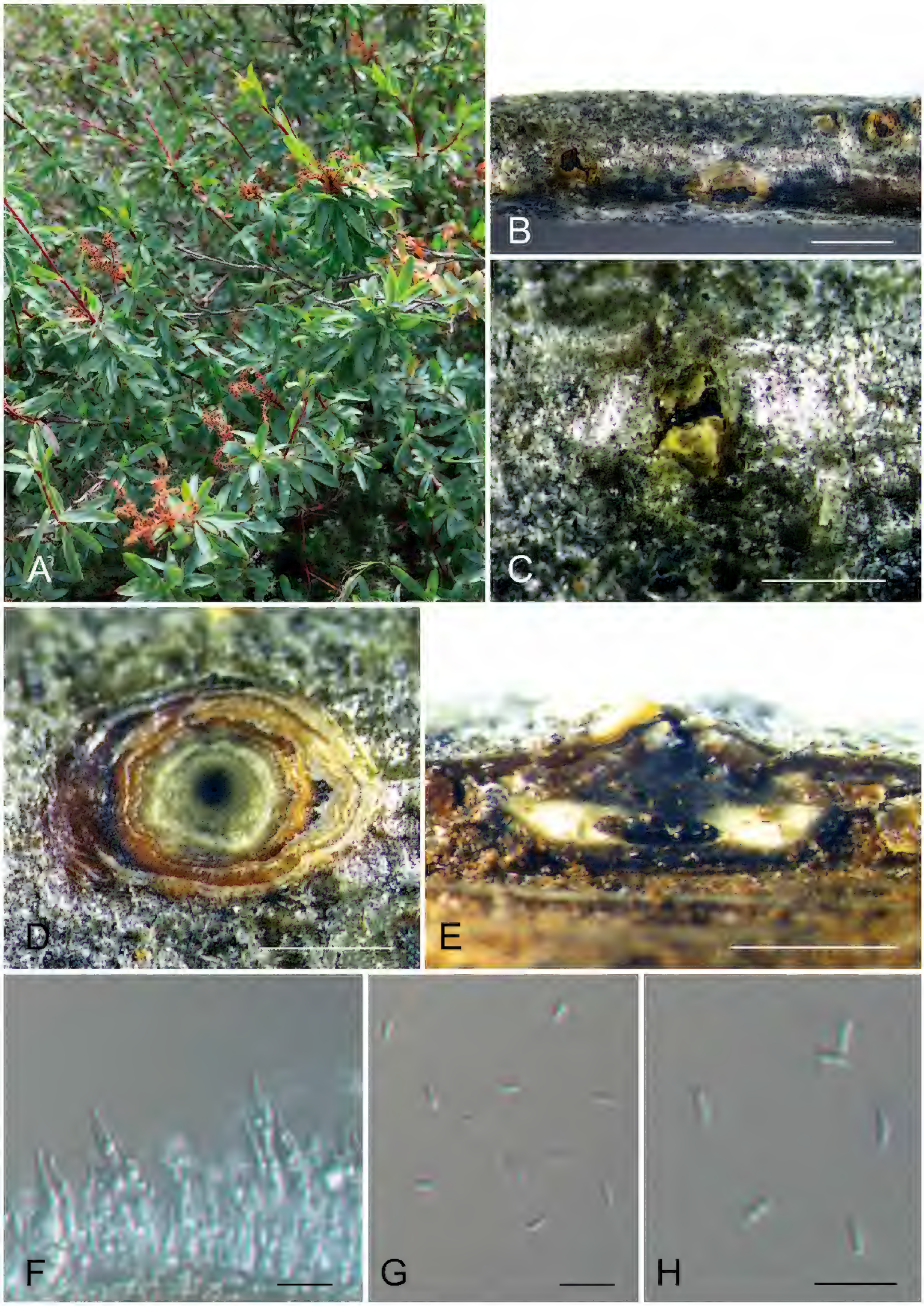

Figure 4. *Cytospora sibiraeicola* (CAF800084, holotype) **A, B** canker disease symptom **C** conidioma **D** transverse section through a conidioma **E** longitudinal section through a conidioma **F** conidiophores and conidia **G, H** conidia. Scale bars: 2000 µm (**B**); 1000 µm (**C–E**); 10 µm (**F–H**).

Discussion

Cytospora is a species-rich genus occurring on various plant hosts (Fotouhifar et al. 2010; Aiello et al. 2019; Jayawardena et al. 2019; Úrbez-Torres et al. 2020; Hanifeh et al. 2022). However, in the third pole of the Earth named Qinghai-Tibet Plateau, canker pathogens, such as *Cytospora*, have been seldom surveyed previously. In the comprehensive study on the genus *Cytospora* in China, only one species *C. chrysosperma* was recorded from Ulmus pumila in Tibet (Fan et al. 2020). Subsequently, *Cytospora cotoneastricola* and *C. tibetensis* from *Cotoneaster* sp. and *Cytospora rosicola* from *Rosa* sp. were discovered in Tibet (Pan et al. 2020). The current study introduces two new species named *C. myricicola* from *Myricaria paniculate* and *C. sibiraeicola* from *Sibiraea angustata* in Tibet, China. In addition, a new host record of *C. populina* on *Prunus cerasifera* was discovered.

To our knowledge, *Cytospora myricicola* is the first species of *Cytospora* discoved on the host genus *Myricaria* (Fan et al. 2020). *Cytospora sibiraeicola* and *C. sibiraeae* have been recorded from the host species *Sibiraea angustata* (Liu et al. 2015). *Cytospora sibiraeae* was described, based only on the sexual morph and is currently impossible to be distinguished from *C. sibiraeicola* morphologically (Liu et al. 2015). However, these two species occurring on *Sibiraea angustata* are phylogenetically obviously distinct (Fig. 1).

Species of *Cytospora* are known as opportunistic pathogens mainly infecting woody hosts and some of the species occur on a wide host range (Adams et al. 2005; Fan et al. 2020). The *Cytospora* species and their host association have been revealed in this study; however, further studies are required to confirm the fungal pathogenicity.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: JRL, JTL, NJ. Methodology: NJ. Formal analysis: JRL. Investigation: JRL, JTL, NJ. Resources: JRL, JTL, NJ. Data Curation: JRL, JTL. Writing - Original draft: JRL. Writing - Review and Editing: NJ. Visualization: JTL, NJ.

Author ORCIDs

Jiangrong Li https://orcid.org/0000-0002-6679-5227
Jieting Li https://orcid.org/0009-0001-8984-7261
Ning Jiang https://orcid.org/0000-0002-9656-8500

Data availability

All of the data that support the findings of this study are available in the main text.

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